Rec'd from 16
10/6/82

HAUGHNESSEY NO. _____ REVIEW NO. _____

EEB BRANCH REVIEW

DATE: IN 7/30/82 OUT OCT 4 1982

ILE OR REG. NO. 3125-GGN, 3125-GGR

ETITION OR EXP. PERMIT NO. _____

AT OF SUBMISSION 7/22/82

ATE RECEIVED BY HED 7/29/82

) REQUESTED COMPLETION DATE 10/8/82

EB ESTIMATED COMPLETION DATE 10/1/82

) ACTION CODE/TYPE OF REVIEW 176/Resubmission - old chemical -- New Food/Feed Use

YPE PRODUCT(S): I, D, H, F, N, R, S Insecticide

ATA ACCESSION NO(S). _____

ROJECT MANAGER NO. W. Miller (16)

RODUCT NAME(S) Oftanol 1.5G: 3125-GGR

Oftanol 5G: 3125-GGN

COMPANY NAME Mobay Chemical Corporation

SUBMISSION PURPOSE Submission of Fish Embryolarvae Study

HAUGHNESSEY NO.	CHEMICAL, & FORMULATION	% A.I.

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM:

TO: William Miller, Product Manager (16)
Registration Division (TS-767)

THRU: Harry Craven, Section Head
Ecological Effects Branch
Hazard Evaluation Division, (TS-769)

THRU: Clayton Bushong, Chief
Ecological Effects Branch
Hazard Evaluation Division, (TS-769)

SUBJECT: Review of fish embroylarval study for completion of environmental hazard assessment of pending application for turf use.

Mobay has submitted an early fish life stage on rainbow trout as requested by the Ecological Effects Branch (EEB) 9/18/81. After review by this office, it was concluded that this study does not comply with the excepted ASTM Guidelines, and will not fulfill EPA Guideline requirements. The major inadequacies of this study were as follows: control mortality too high for swim-ups; lack of data pertaining to growth of fish; no carrier control (refer to Reviewers Evaluation in the accompaning Data Evaluation Review).

Although this test will not support registration, some information can be derived from the larval exposure. At concentration levels between 66.1 and 206 ppb, significant mortality occured to fish larvae. These results and the calculated Estimated Environmental Concentration (EEC) for turf (EFB's calculation was .21-.23 ppm) suggest that there is an unreasonable risk to fish. Mobay should be informed that, according to this information, they have exceeded the RPAR criteria for potential chronic effects with this pesticide.

Michael Rexrode
Fishery Biologist
Ecological Effects Branch

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Chemical: Oftanol; Isofenphos

Citation: Carlisle, J.C., "Isofenphos Toxicity to Rainbow Trout Early Life Stages," prepared by Mobay Chemical Corporation, Stanley Research Center, Stilwell, Kansas.

Reviewed by: Miachel Rexrode, Fishery Biologist
Ecological Effects Branch
Hazard Evaluation Division (TS-769)

Date Reviewed: September 23, 1982

Test Type: Early Life Stage Fish Study

Test Species: Rainbow Trout (Salmo gairdneri)
Test Material: Isofenphos (91.9% a.i.)

Reported Results: The toxicity of isofenphos (oftanal) to early life stages of rainbow trout was tested in a 28-day exposure of embryos and larvae. No concentration-related embryonic death was observed. At concentrations of 206 and 518 ppb, significant mortality occurred in the exposed larvae. At 7, 21, and 66 ppb, mortality was not significantly greater than in the controls. The 28-day LC_{50} (with 95% confidence limits) was 168 (152-185) ppb.

Materials/Results/Procedures

Tests Procedures -

Rainbow trout eggs incubated to eyed stage, were acclimated to the test temperature over a five-day period.

Each of six test vessels consisted of a 5-gallon, linear polyethylene, cylindrical bucket with a nylon-screened overflow hole at the 15-liter level. Within each test vessel were four incubation chambers, each consisting of a 6-inch long X 3-inch diameter section of polyvinyl chloride pipe with a polyethylene screen which supported the eggs 3 inches from the bottom and several 3/8-inch screened holes within one inch of the top. Four 3/4-inch plastic tubes supported a plexiglass plate which, in turn, supported the chambers. An airstone in each tube created a current which flowed up through the tubes and down through the incubation chambers. A cooled, circulating water bath kept the temperature in each chamber within the range 11.0 to 13.1°C.

Carbon-dechlorinated tap water was used at a flow rate of 75 liters per day per test vessel. The chemical profile of this water was determined by analysis of weekly samples taken from each test vessel. The results of these analyses are found in Appendix I.

Temperature and dissolved oxygen content of the test solutions were measured daily by inserting an oxygen/temperature probe into each test chamber. The minimum dissolved oxygen concentration (measured by this method) was 8.7 mg/l. The mean incubation temperature for each test chamber is given in Table II. The maximum variation between chambers was 1.4°C.

A stock solution was prepared using 1.0 mg isofenphos and 0.1 ml acetone per liter. A solenoid valve diluter system was used to provide nominal concentrations of 0, 10, 30, 90, 270 and 810 ppb of the test substance. A container to promote mixing of toxicant-bearing water and dilution water was used between the diluter and the test vessels. The diluter cycle time was ten minutes with complete replacement every five hours. The concentrations were verified by chemical analysis on days 0, 3, 7, 14, 21, and 28 of the test. The photoperiod was 16 hours of light and 8 hours of darkness controlled by a Tork Timer.

For each concentration 120 eggs were used, divided between four test chambers. Deach fish were removed and counted daily. Hatched fry and swim-ups were counted daily.

Analytical Procedures

The water solution was extracted three times with dichloromethane. The combined extracts were evaporated to dryness and diluted with ethyl acetate for gas chromatographic analysis using a flame photometric detector in the phosphorus mode. Recoveries of isofenphos at 10, 30, 90, 270 and 810 ppb ranged from 92.6 to 104%.

Statistical Procedures

The cumulative mortality in each concentration was compared with that in the control group using a one-tailed student's T test for the difference between sample means. For dose-response analysis, cumulative mortality in each test concentration was adjusted for the mortality level in the control group according to the formula:

$$P_T' = \frac{P_T - P_C}{1 - P_C}$$

(Abbot, W. S. 1925, "A Method of Computing the Effectiveness of an Insecticide," J. Econ. Ent. 18:265-267). The adjusted mortality data (P_T') were then analyzed by computerized probit anlaysis using a program provided by SAS Institute, Inc., Box 8000, Cary, N. C. 27511.

RESULTS AND DISCUSSION

Chemical Analysis

Results of chemical analysis of the test solutions are given in Table I. The mean concentrations ranged from 64 to 76% of the nominal concentrations. Since the observed concentrations were sonsistently lower than nominal values, the former were used in toxicity calculation.

Hatching

The incubation periods (from the beginning of the study) along with percent hatch are given for each concentration in Table II. While there was no concentration-related effect on hatching success, incubation time was decreased as higher concentrations. Since this effect was observed only at lethal levels, it is not considered biologically significant.

Mortality

Mortality data are summarized in Table III. Cumulative mortality is presented graphically in Figure 1. Mortality began to rise above background levels on day 7 for the highest test concentration and on subsequent days for lower concentrations.

Twenty-eight day cumulative mortality was significantly greater than control mortality for concentrations 206 and 518 ppb (analytical) Median lethal concentration (LC_{50}) (and 95% confidence limits) was 168 (152-185) ppb. The equation for the mortality (y) versus concentration (x) curve was:

$$y \text{ (probit units)} = -5.64 + 4.78 \log x \text{ (ppb)}.$$

Clinical signs observed included persistent swollen, edematous yolk sacs, and depression and pigment abnormalities in most fish before death.

Appendix I. Results of Water Chemistry Analysis

Component	Day 0	Day 7	Day 14	Day 21	Day 28
pH	8.9 (8.8-9.0)	9.2 (9.1-9.3)	9.0 (8.9-9.0)	9.5 (9.5)	8.5 (7.9-9.1)
Dissolved O_2 (mg/l)	10.2 (10.0-10.3)	9.5 (9.5-9.6)	9.4 (9.2-9.8)	10.3 (10.1-10.5)	10.6 (10.2-10.8)
Hardness (mg/l as $CaCO_3$)	134 (133-135)	174 (173-174)	168 (166-169)	188 (187-189)	95 (91-98)
Alkalinity (mg/l as $CaCO_3$)	59 (58-59)	51 (51-52)	73 (71-74)	64 (63-65)	71 (68-75)

Each value is the mean (and range) of 6 measurements.

Table I. Isofenphos Analytical Results

Concentration ppb

Nominal Concentration	Day 0	Day 7	Day 14	Day 21	Day 28	Mean	S.D.	Mean/ Nominal
0	1.86	1.40	0.01	0.13	0.00	.652	.907	-
10	6.91	7.50	7.80	6.13	6.23	6.91	.743	.69
30	13.7	19.7	25.3	20.1	25.4	20.8	4.84	.69
90	50.9	74.0	63.9	63.9	77.7	66.1	10.5	.73
270	140	213	243	204	228	206	39.6	.76
810	373	459	523	548	684	518	115	.64

Table II. Hatching Summary

Concentration	(A) Mean Incubation Time	(B) Mean Incubation Temperature	Degree Days (A X B)	Total Hatch
0	8.49 days	11.91°C	101.1	117
10	8.34 days	11.90°C	99.24	117
30	8.28 days	11.93°C	98.91	116
90	8.18 days	12.0°C	98.16	112
270	7.56 days	12.17°C	92.03	117
810	6.27 days	12.42°C	77.86	113

Table III. Mortality Summary

Group	Embryo Mort.	Larval Mort.	Total Mort.	Survivors	Adjusted Mort. (P_T')	Swim-ups	Swim-ups (% of Survivors)
0	3	8	11	109	0	60	55
10	3	17	20	100	0.083	48	48
30	4	7	11	109	0[2]	100	92
90	8	7	15	105	0.018[2]	88	84
270	3	83	86[1]	34	0.688[2]	8	24
810	7	111	118[1]	2	0.981[2]	0	0

[1] Significantly different from control
[2] Used in Probit Analysis

Reviewers Evaluation:

This test does not comply with the ASTM Guidelines, for a fish early life stages study. This study will not fulfill EPA Guideline requirements.

The significant short-comings of this study concern the survival rate of negative controls, lack of carrier controls and the lack of information regarding fish growth. Swim-up percent survivors was 55% in the negative control, suggesting excess control mortality and possible water contamination or inproper laboratory procedure. Acetone was used as the chemical ccarrier, but, no acetone control was set up. The effects on larval growth are concentration - dependent, however, no data was submitted regarding this paramater (i.e. length - weight).

According to ASTM Guidelines, discrete variables (e.g. numbe hatching or surviving) should be analyzed using some form of a 2x2 contingency table. Excessive control mortality makes biological interpretation of the swim-up data highly questionable. Can be minimized if either glass, No. 316 stainless steel, polyamide (nylon) or fluoroplasties are used. Mobay's study indicated that "each of the six test vessels consisted of a 5-gallon, linear polyethylene cylindrical bucket --" and that the incubation chambers each contained polyvinyl chloride pipe and polyethylene screen (which supported the eggs). The polyethylene and polyvinyl chloride adsorb chemicals readily.

According to ASTM, concentration levels and controls should be set-up in duplicate. This was not done in the Mobay study.

Category: Supplemental

Repairability: N.A. ; control mortality too high, no data on growth, no carrier control